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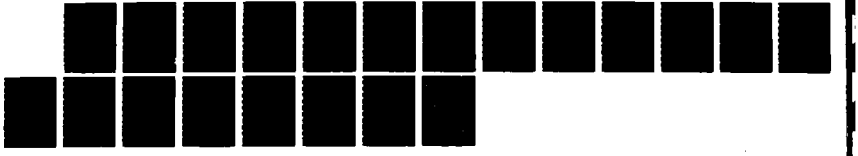

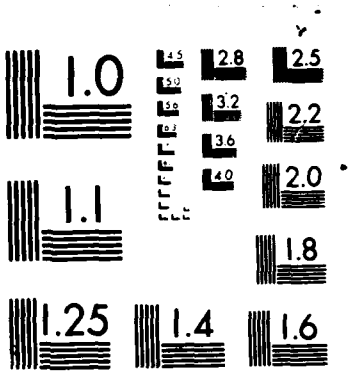

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The proposed work has been directed to determination of the structure of acetylcholinesterase. During the project period we have determined the primary structure of the Torpedo enzyme through amino acid sequencing and the isolation and sequencing of a c-DNA clone encoding for the 11S form of the enzyme. Peptides corresponding to the active center of the enzyme and a C-terminal region have been synthesized and antibodies are being raised for the purpose of detecting the phosphorylated enzyme and delineating functional regions of the molecule. Finally, progress has been made on the secondary structure of the enzyme.

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THE PRIMARY SEQUENCE OF ACETYLCHOLINESTERASE AND SELECTIVE ANTIBODIES FOR THE DETECTION OF ORGANOPHOSPHATE TOXICITY

ANNUAL REPORT
PALMER TAYLOR, Ph.D.

DECEMBER 3, 1985

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SUMMARY

The proposed work has been directed to determination of the structure of acetylcholinesterase. During the project period we have determined the primary structure of the Torpedo enzyme through amino acid sequencing and the isolation and sequencing of a c-DNA clone encoding for the 11S form of the enzyme. Peptides corresponding to the active center of the enzyme and a C-terminal region have been synthesized and antibodies are being raised for the purpose of detecting the phosphorylated enzyme and delineating functional regions of the molecule.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and use of Laboratory Animals and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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Introduction

During this period we have completed the primary structure of acetylcholinesterase through amino acid sequencing and c-DNA cloning and sequencing. This information should prove important to the many groups working on acetylcholinesterase structure.

Background

The high turnover number of acetylcholinesterase and availability of selective inhibitors have allowed extensive study of its distribution, catalytic mechanism, and functional role in cholinergic neurotransmission. The recent elucidation of the primary structure of the enzyme through protein chemistry and the isolation of its gene have added a molecular dimension to continuing studies on this protein, which controls the residence time of acetylcholine in the synapse. Acetylcholinesterase exhibits an extensive polymorphism of structure and, since the catalytic parameters of the individual enzyme species are largely invariant, the structural diversity appears critical only to the regulation of the cellular disposition of this molecule. Recent structural studies clearly show that acetylcholinesterase behaves as a secreted rather than an integral membrane protein. The post-translational modifications provide an appropriate link to tether the enzyme to specific extracellular locations. Being an extracellular enzyme, modifications of structure critical to its disposition should occur prior to export to its site of residence. Thus, variations in structure responsible for cellular localization must either be encoded in the genome or be differentially affected by posttranslational events of biosynthesis.

Acetylcholinesterase Polymorphism

Since the initial finding of Massoulié and Reiger (1) that a native form of acetylcholinesterase contains an elongated tail unit linked to defined number of catalytic subunits, the control of individual species of acetylcholinesterase in relation to innervation, developmental processes and activity of excitable cells has received considerable attention (2). Two general classes of acetylcholinesterase species exist. The most unique is the elongated or dimensionally asymmetric species, which contains a filamentous tail unit disulfied-linked to tetrametic sets of catalytic subunits. The tail unit contains a collagen-like sequence distal to the catalytic subunits. Each strand of the triple helix is joined to a tetramer of catalytic subunits. Since each catalytic subunit is approximately 70,000 daltons, elongated species close to a molecular weight of one million are generated. In the case of Torpedo, but not Electrophorus, a second type of structural subunit has been identified as a non-collagenous, 100,000 daltoon peptide (3). It will be of interest if this structural entity also prevails in higher species. Treatment of the asymmetric form with collagenase markedly shortens the tail unit and a light tryptic digestion will remove the structural subunits without apparently altering catalytic parameters or the structure of the catalytic subunit (cf. 2). The asymmetric species appear to be fully assembled in the Golgi apparatus prior to export from the cell (4,5).

The second class are the globular forms, which show considerable structural variegation in subunit assembly (monomers to tetramers) and in hydrophobicity. The hydrophobic forms identified to date result from the cotranslational addition of glycophospholipid to the C-terminal carboxyl group of the nascent peptide chain (6,7). This modification resembles that seen in the variable surface glycoprotein of trypanosomes and the Thy-1 antigen (8). It is quite possible that the nature of the glycophospholipid additions are not identical in the various tissues and may, in themselves, provide a basis for microscopic regional localization. Hence, the globular forms range from totally soluble species to species with particular hydrophobic glycophospholipids conjugated to the peptide chain.

Methods

The methods used for generation of the data described below have been documented in our manuscripts now published in the open literature and will only be described briefly.

A. Determination of the primary structure of acetylcholinesterase Primary structure determinations relied on both tryptic and CNBr fragmentation. The peptides were initially size separated on Sephadex G-50 and then subjected to reverse phase HPLC on C-18 or C-4 columns. Sequencing initially involved dansyl-Edman and the Spinning cup, but after the first 6 months of the contract employed the gas phase method. Details may be found in (9).

B. Antibody Generation and Assessment of Reactivity. Both monoclonal and polyclonal antibodies were made to the 11S and 5.6S species of acetylcholinesterase. Similar methods were used for generation of antibodies to the individual peptides. Antibody reactivity and titers were determined by the enzyme-linked immunoassay (ELISA) method and by radioimmunoassay using ^{125}I-acetylcholinesterase. Details may be found in references 3 and 10.

C. Cloning and Sequencing of a cDNA-clone Encoding the 11S Species of Acetylcholinesterase. As described in reference 11, nucleotide probes to tandem sequences contained within a CNBr peptide were used to hybridize recombinants in a -gt 10 library. Positive clones were isolated, tested by hybridization and sequenced using M-13 sequencing vectors. Details are found in reference 11.

Results

1. Amino acid sequencing of Torpedo acetylcholinesterase

Our sequencing strategy is designed to fulfill several objectives:

a. To obtain a sequence sufficient for the design of multiple nucleotide probes for c-DNA library sequencing.

b. To employ the sequence to verify inferred amino acid sequence resulting for c-DNA sequencing and correlate the c-DNA sequences with the multiple acetylcholinesterase gene products.

c. To elucidate differences in sequence between the molecular forms of acetylcholinesterase.

d. To identify critical regions in the molecule: active center, chemically modified residues, N-terminal sequence, C-terminal sequence, glycosylation sites, cysteine-containing peptides and other sites of post-translational modification.

e. To provide a peptide fractionation scheme by which other cholinesterases of lower abundance can be sequenced and homologous regions identified.

To date, we have sequenced about 80% of the tryptic peptides of the 11S enzyme and 30% of the tryptic peptides of the 5.6S enzyme. All of the CNBr peptides have been isolated from the 11S enzyme and ~30% sequenced. A smaller number have been sequenced in the 5.6S enzyme. The sequences are summarized in Table 1. Several findings should be highlighted:

a. Large tryptic peptides for the active center (24aa) were isolated and sequenced. Sequence was verified by chymotryptic digested and the position of this peptide in the whole enzyme could later be verified. The active center serine is serine 200. These peptides are identical in the 11S and 5.6S species (1) (fig. 1).

b. The N-terminal peptides of the 11S and 5.6S enzymes were identified and sequenced through 42 and 30 residues, respectively. These peptides were also identical in the 11S and 5.6S species. These sequences were later verified by the c-DNA sequence and, more important, a leader peptide was demonstrated for the unprocessed acetylcholinesterase. Cleavage occurred C-terminal to an Ala, giving rise to the N-terminal Asp residue in the processed protein. A candidate C-terminal tryptic peptide ending in leucine was also identified in the 11S species. That this peptide was a C-terminal tryptic peptide was later verified by finding a stop codon at amino acid position 575, which followed the leucine code and thus ended the open reading frame on our cloned cDNA. An analogous C-terminal peptide has not been found for the 5.6S enzyme, and we believe a posttranslational modification occurred here, providing one of the points of structural departure of the two enzyme forms.

c. The cysteine-containing peptides were identified by reduction and subsequent alkylation by [^{14}C]iodoacetate. We obtained more cysteine peptides than would be predicted by the c-DNA sequence, but they arose simply from incomplete cleavages. All of these peptides can be placed in the inferred amino acid sequence on the basis of either their total sequence of their N-terminal residue identification and partial sequences. We have initiated fractionations of the unreduced enzyme with the essential aim of establishing the positions of the inter- and intrasubunit disulfied bridges. One of the eight cysteines appears to exist as a free sulfhydryl group and has been labeled with bimane in the 5.6S enzyme. Isolation and sequencing of the peptide reveals that the cysteine is at position 232.

Sites of glycosylation have been identified by lectin blotting of the individual peptides and by broad elution profiles that reflect microscopic heterogeneity within the peaks and their coalescence following endoglycosidase F treatment. An example is shown in fig. 2. Three of the four potential N-linked glycosylation sites have been located by peptide isolation (asparagine positions 56, 457, and 533), while it appears that position

TABLE I: Sequences of Torpedo californica Acetylcholinesterase Peptides*

11S Acetylcholinesterase

I46	ivgywa2fa-c
I77	vpvegcvfanef-nnci
III88	fsivpvddgqfw(yst)k
II61	kpwsgvw-asnyp (carbohydrate and CM cysteine)
II61	kpwigvwfhnypl
IV33	dnhsellvntksgkvmgtrvpvlsshisafl givfaeqvgidv (N-terminal)
IV67	tvtifgesaggasvgmhilspgsr (active site)
IV14	tgnpneptsqesk
IV26	le-ea
IV62	fgbgtyly-pdt--yr
IV63	ailqsg-vdcepa
I46	ivgywaa2fa-c
I77	vpvegcvfanef-nnci
VI87	vqvcwfwnqflp
VII17	rpepk
V02	fidlntepmnk
V04	galqwvhdniqffggdpmk
I67	iteahh
II49	nlbbglncnl-nsaeelihicl
III65	-(av)dedcly-niwspgca
IV69	v-afalig
II67	l2vphandlgld(5)v(g)lqytdwmddnngik
IV26	hescael (c-term)
II61	kpw(i)gvw-as(n)ypl (carbohydrate, CM cysteine)
I46	ivgywa2fa-(c)I77
I77	v(p)vegcvfane(f)(lp)nnci
III88	f(s)ivpv(d)dgqfw(ystk)
II63	dglddivgbhnvicplmhf
II62	kpwphawdlg-p
II64	lsvphandlgldlvt
II101	dhnlvwpew-gvi(h)gyei--g-l-p
II68	lsvphandlgldtvglqytdwmd(ing) 4e7 immunoreactive)
I73	v(aph)vegcvfane(yf)(lp)(np)nc(f)-(hg)v(if)e
II49	nlbbglncnl-nsagglihicl (carbo)
III59	-(av)(sd)edcly-niw(s)pgca
IV57	ailqsgspncpwasvsv(aZg)r
VI87	vq(v)cxfwnqflp
I61	kpw(l)gvw(f)(h)(n)y(p)l
I73	v-vegcvfane--ncf--v(ip)g
III64	b-dedcly-niw-pgc
V65	ailqsgspncpwasvsv(azg)r
V01	tgnpnephsqesk
II54	lgvp-a
IV64	aieag
IV71	tvtifg-s

11S Acetylcholinesterase (includes CNBr peptides)

bpd1	mwnpdre(p)
bpd2	mnfvsnyypfgpgvylyflsieapd
bt1	mddnngiknrdglddivgdhnvicplm
bt2	m-wfg-p-pepgkpwngv-was--y-n
bt3	mlntgnfkk(s)qillgvn(yk)(s)fgif(f) lyga(v)(g3)f
bt4	mhvwatfaktgnpnep-eg-(t)kwplifik-(fq)-(e)
VI87	vq-cwfwnqflp
VII17	rpepk
V01	tgnpnephsqesk
V02	fidlntepmnk
V03	ailqsgspncpwasvsv
V04	galqwvhdniqffggdpmk
III65	--dedcly-niw-pgc
II61	pw-gv--a--vpl
IV26	hescael (C-terminal peptide)
I77	v-vegcvfane--nnci
II01	d-nlvwpew-gvi-gy
II49	dlbbglncnl-nsaeeli--cl
II59	lgvpda
II67	l-vphandlgldtvglqytdwmd
III88	f-ivpv-dgqfw
IV14	tgnpneptsqe
IV62	fgdgtyly
IV64	aieag
IV71	tvtifg-s
I46	ivgywa-fa
I67	iteah
IV69	v-afali

5.6 Acetylcholinesterase

IV18	tgnpnep
IX02	gpha-a
VI64	ail-e--pncpwatv-va
	dnhsqllvntksgkvmgt (N-terminal)
	tvtifgesaggasvgmhilspgsr (active site)

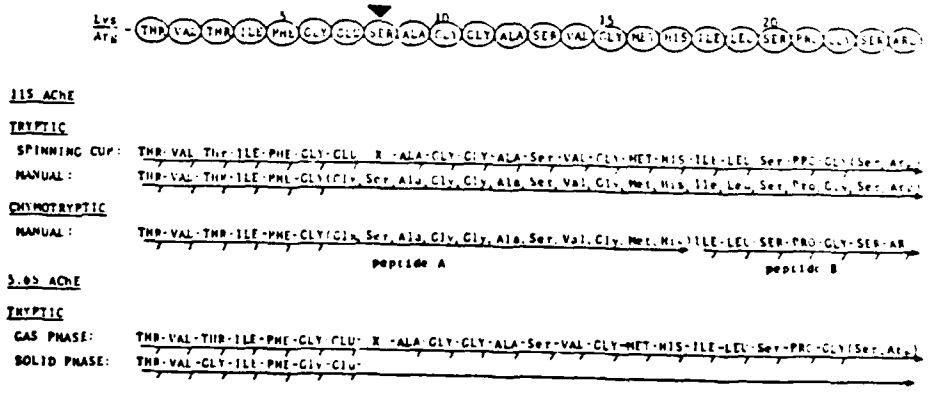
5.6S Acetylcholinesterase

dnhsqllvntksgkv-gt (N-terminal)
IV67 tvtifg
II63 dglddivgdhnvicplmhf
II64 12vphandlgl(dw)avt
VI64 a(i)lqsgsp(ns)cpwatv-va
IV62-64 fgbgtyly(f)(f)n(h)r
IV18 tgnpnep(p)vzeq
IX02 gpha-a
IV64 aigag(a)(v)ae(pg)g-(v)-ppd
II54 lg(v)(p)s(la)a--(dv)
I109 (53)vdl(1)
II59 lgvpda----d--vp
VI64 a-lqsg(s)pncpw
I109 (te)vdl(1)
II59 lgvpda----d--vp
VI64 a-lqsg(s)pncpw
II67 lgvphad-dg
VI64 ailqsgsp(ds)cpwatv-va

11S Acetylcholinesterase: CNBr Peptides

bt1 mddnngiknrdgldd ivgdhnvicp
bt2 m-wfg-p-pepgkpwngv
bt3 mlntgnfkk-qillgvn--fgif-lyga
bt4 mhvwatfaktgnpnep-eg--kwplifik
bd1 mwnpdre
bd2 mnrvsnyypfgpgvylyflsieapd

*bt and bd peptides are CNBr peptides. Roman numerals denote Sephadex fractions of the initial fractionation and Arabic numbers denote the peak fraction from high-pressure liquid chromatography.



Sequence analyses of the active site tryptic peptides and chymotryptic peptide. The *arrow* indicates the [^{3}H]isopropylphosphoryl-labeled serine. The consensus sequence is indicated in *circles* at the *top*. Residues in *parentheses* indicate placement only by amino acid composition. Residues indicated by *capital letters* are positions identified unambiguously, whereas sequences in *lower case letters* were tentatively identified. *AChE*, acetylcholinesterase.

Fig. 1 Active center sequences of the 11S and 5.6S acetylcholinesterases.

416, despite the presence of an Asn, X, Ser/Thr, is not glycosylated. Overall carbohydrate compositions suggest that we may have an O-linked site, but this remains to be established.

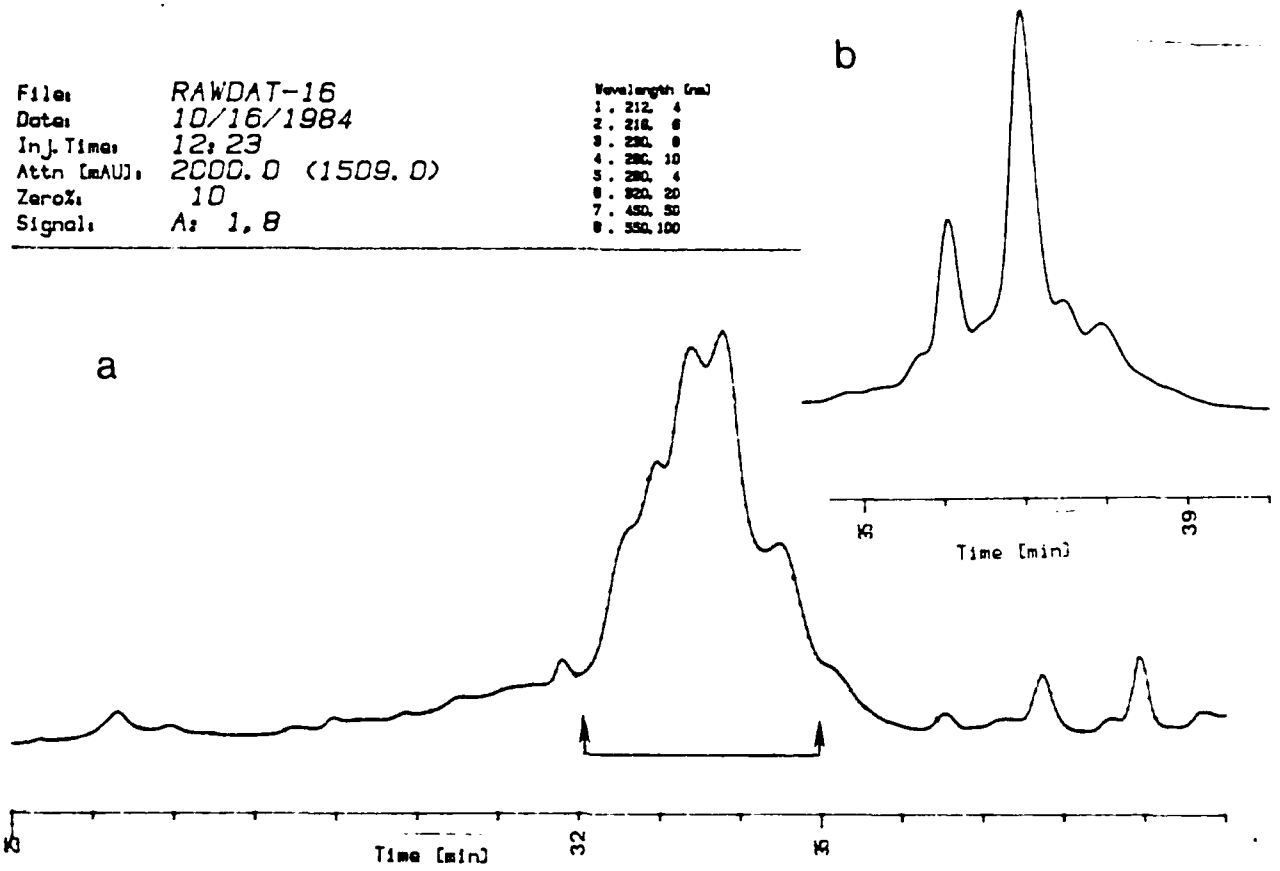


Figure 2: HPLC Profiles of 11S acetylcholinesterase peptides prior to (a) and following (b) endoglycosidase F treatment. Fractions 32-35 were isolated, lyophilized and treated with endoglycosidase F. The fractions were run on the same column (C-18 reverse phase), using an identical trifluoroacetic acid-H_2O-acetonitrile gradient. The elution profile with its altered elution positions and decreased complexity is shown in the inset.

d. Potential sites that serve as epitopes for the monoclonal antibodies raised by B.P. Doctor have also been identified. The two of particular interest are 4E-7 and AE-2. 4E-7 reacts selectively with the 5.6S enzyme (2) and has been found to react only with the glycosylated form

of the enzyme. Treatment with endoglycosidase F but not endoglycosidase H eliminates the antigenicity. 4E-7 reacts equally well with the native and denatured enzyme. A peptide extending between residues 358 and 386 shows the greatest reactivity with 4E-7 as determined by antibody blotting and competitive immunoprecipitation. We expect this peptide to be one of those unique to the 5.6S enzyme.

The other antibody of interest is AE-2, an antibody isolated by Fambrough and colleagues (3) which shows considerable species cross-reactivity. AE2 was found to react with a peptide found by B.P. Doctor in fetal calf serum acetylcholinesterase. This peptide has been found between positions 12 and 18 and considerable homology between species exists in a large portion of this peptide (cf: Table III). Several other antibodies are less well characterized. However, some, such as 4G-7 and 2C-9, show high titers and good immunoprecipitation capacity.

The rather brief description given here describes the bulk of the studies performed during the past 2 years. Extensive fractionation and sequencing were required to achieve this state of progress for an enzyme subunit size of 575 amino acids which exists in multiple enzyme forms. These endeavors have been very much facilitated by the instrumentation provided in the contract. Our basic sequencing strategy was to reduce and alkylate the protein with [^{14}C]iodoacetate and size-fractions were collected which were then subjected to reverse phase HPLC on C_4 columns, using an acetonitrile-1% aqueous trifluoroacetic acid gradient. Peptides that fractionated poorly on C_{18} columns usually resolved well on C_4 columns. Compositions and N-terminals were ascertained before subjecting the peptides to gas phase sequencing. Profiles of some of the many fractionations can be found in MacPhee-Quigley et al. (9).

2. Preparation of antibodies directed to the active venter for acetylcholinesterase

Having obtained the active center peptide sequence, we then synthesized a 25 mer peptide to generate antibodies to the active center of acetylcholinesterase. The peptide was synthesized by the Merrifield solid phase methods, using Dr. Russell Doolittle's facility, Department of Chemistry, University of California, San Diego. An N-terminal lysine was added to promote solubility, giving the sequence:

Lys-Thr-Val-Thr-Ile-Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ser-Val-Gly-Met-Ile-Leu-Ser-Pro-Gly-Ser-Arg.

Antibodies are being generated in three ways: Monoclonals are being raised by B.P. Doctor and M.K. Gentry at Walter Reed. The fusions are now complete, positives colonies have been selected, and we should be screening for precise titers and selectivity next month. Polyclonal antibodies are also being raised in rabbits at San Diego, using two forms of immunogens: the peptide dispersed in liposomes and the peptide conjugated to hemocyanin. The monoclonal antibodies will have the potential of obtaining isolated antibodies directed to small peptidic domains, some of which show little species cross-reactivity. Other, owing to extensive homology, will exhibit considerable species cross-reactivity. The polyclonal antibodies can be expected to have the higher titers and will prove most useful for screening in vitro translation products and the development of highly sensitive assays

for the active center of acetylcholinesterase. Antibodies to synthetic peptides have the advantage of not showing cross-reactivity to contaminant proteins in biological preparations.

3. Comparative sequencing of Torpedo and other cholinesterases

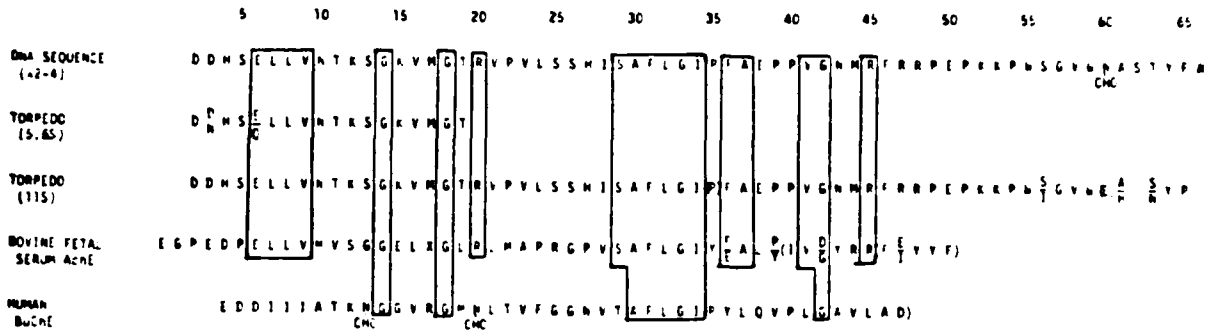
Our initial findings showing extensive homology of the active center peptides of Torpedo acetylcholinesterase and human butyrylcholinesterase (Table II) and the substantial homology in the N-terminal region of the two proteins prompted a further homology search in conjunction with Drs. Oksana Lockridge and Bert LaDu at the University of Michigan, and we see extensive similarity throughout the two molecules. Several peptides showing corresponding sequences can readily be found if our peptides (Table I) and their peptides are compared. The Michigan group also has about 80% of the peptides sequenced and with a total inferred sequence available in Torpedo, it should be possible for them to place the remaining peptides within the linear sequence.

TABLE II Sequences Of Active Site Regions

	5							P		10						15							20
TORPEDO ACETYLCHOLINESTERASE	NH_2 - THR	VAL	THR	ILE	PHE	GLY	GLU	SER	ALA	GLY	GLY	ALA	SER	VAL	GLY	MET	HIS	ILE	LEU	SER			
EEL ACETYLCHOLINESTERASE						GLY	GLU	SER	SER	GLU	GLY	ALA	ALA	GLY									
HUMAN PSEUDOCHOLINESTERASE	NH_2 - SER	VAL	THR	LEU	PHE	GLY	GLU	SER	ALA	GLY	ALA	ALA	SER	VAL	SER	LEU	HIS	LEU	LEU	SER			
EQUINE PSEUDOCHOLINESTERASE					PHE	GLY	GLU	SER	ALA	GLY	SER	ALA	ALA										
EQUINE ALIESTERASE					PHE	GLY	GLU	SER	ALA	GLY	ALA	ALA	SER										
BOVINE TRYPSINOGEN	LYS	ASP	SER	CYS	GLN	GLY	ASP	SER	GLY	GLY	PRO	VAL	VAL	CYS	SER	GLY	LYS						
PORCINE TRYPSIN	LYS	ASP	SER	CYS	GLN	GLY	ASP	SER	GLY	GLY	PRO	VAL	VAL	CYS	ASN	GLY	GLN						
S. GRICEUS TRYPSIN	VAL	ASP	THR	CYS	GLN	GLY	ASP	SER	GLY	GLY	PRO	MET	PHE	ARG	LYS	ASP	ASN						
E. COLI ALKALINE PHOSPHATASE	LYS	PRO	ASP	TYR	VAL	THR	ASP	SER	ALA	ALA	SER	ALA	THR	ALA	TRP	SER	THR						

Human butyrylcholinesterase and Torpedo acetylcholinesterase can be expected to diverge on a phylogenetic basis and the basis of distinct enzymatic properties (i.e., the butyrylcholinesterase will accommodate substrates with large acyl groups, it does not show substrate inhibition and it is preferentially inhibited by different alkylphosphates). Therefore, one might expect that other mammalian acetylcholinesterases will possess structures showing structural divergence between these two limiting cases. In this regard, the fetal bovine serum acetylcholinesterase has proven useful. The trend in sequence divergence that we might expect can be seen in examining the N-terminal region of four cholinesterases (Table III). A more complete analysis of this mature should prove very useful in identifying various functional and antigenically cross-reactive regions.

Table III N-Terminal Sequences Of The Cholinesterases



4. Isolation of c-DNA clones encoding for acetylcholinesterase

Although this portion of the work was initiated and sustained with the support of the National Institutes of Health, the protein chemistry and molecular biological approaches are integrally linked, and it would have been impossible to proceed as rapidly without having both approaches in the same laboratory. Our library screening employed strategems that relies on hybridization with tandem but not overlapping probes, since we initially found that screening with a single probe yielded a very high incidence of false positives. When sequenced, the false positives were found to be repeating sequences of ~500 bp with rather good base matches (14 of the 17 bases in the mixed probe). The tandem probes eliminated this artifact and were preferable to using probes coding for separate peptides. The latter approach will miss short length sequences. The tandem probe approach usually requires that more amino acid sequence be known, since rather long peptidic stretches are usually required to minimize code redundancy in the probes. Positives to both tandem probes were then screened to a probe encoding for the N-terminal region. This reduced the number of positives and enhanced the likelihood of obtaining full-length inserts. By this approach we have now obtained 7 inserts which clearly encode for acetyl-cholinesterase and 13 more candidates. Their lengths and locations of Eco R1 sites are detailed in fig. 3. Only lambda 2-4 (AchE-1) has been fully sequenced. The sequencing strategy (Fig. 4) and sequence (Fig. 5) are shown.

Acetylcholinesterase c-DNA Clones

(Eco R1 and Hind III Indicate Restriction Sites)

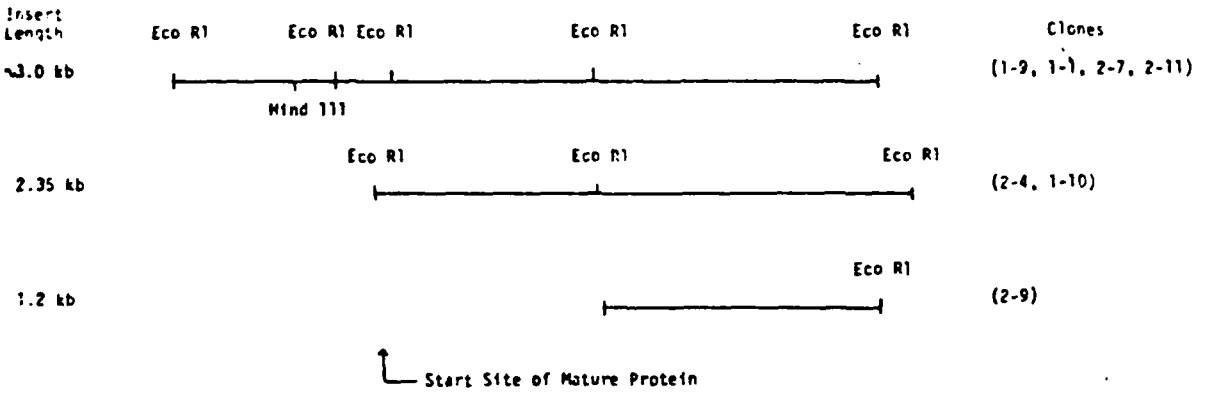


Figure 3: Characterization of several clones encoding for acetylcholinesterase. The length of the clones, Eco R1 and Hind III sites are shown.

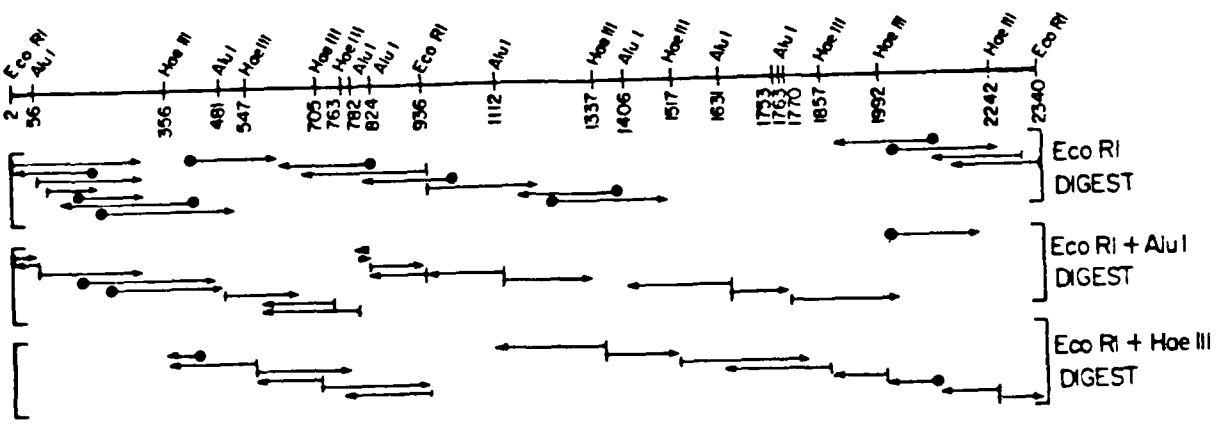


Figure 4: Sequencing strategy for acetylcholinesterase clone AchE-1 (γ2-4). Critical restriction sites (Eco R1, Hae III, Alu 1) and sequencing primer sites are shown.

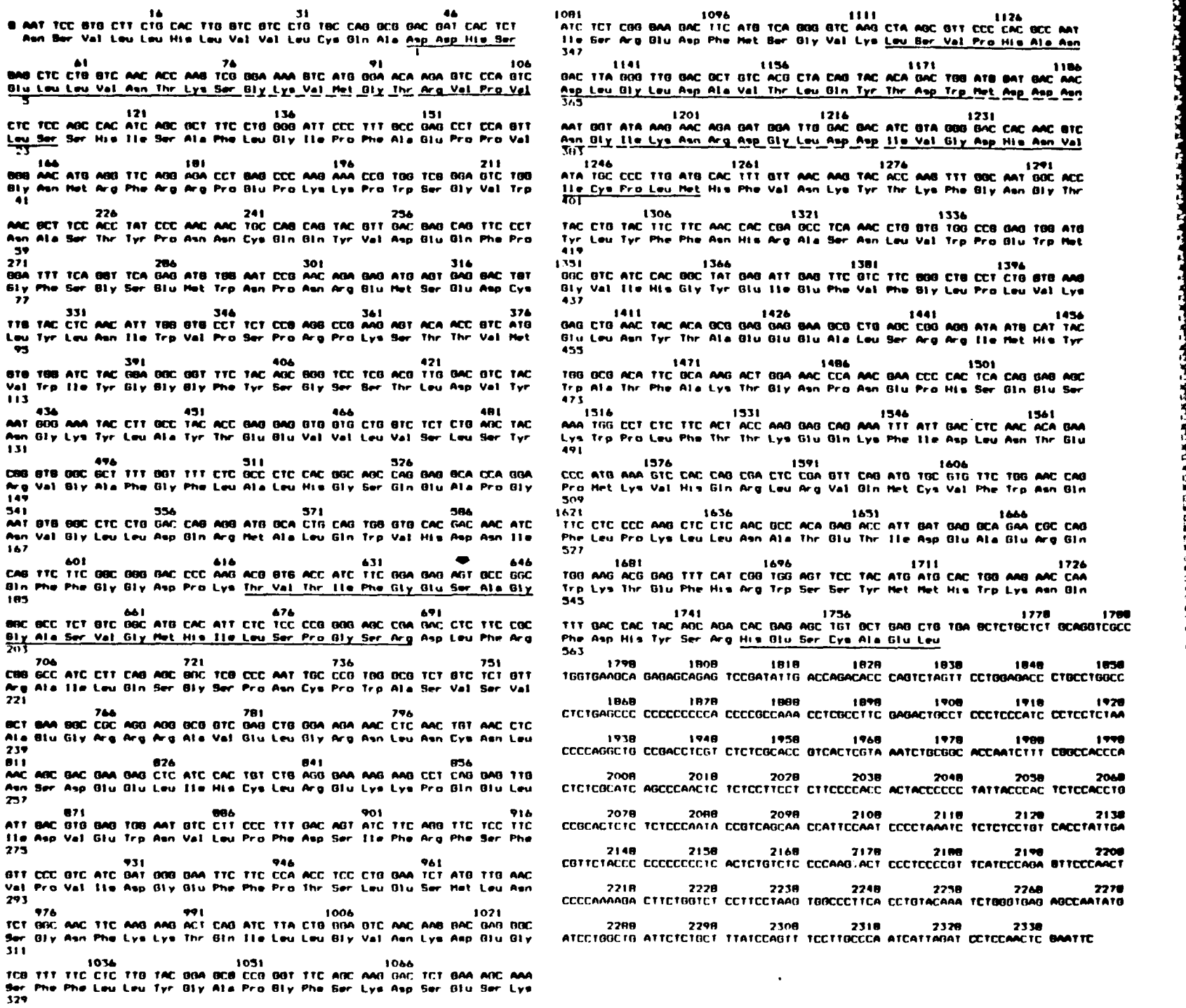


Figure 5: c-DNA and inferred amino acid sequence of clone 2-4.

The insert begins with a 16 amino acid leader peptide, extends through the 575 amino acids of the processed protein, and contains another 570 bases in a 3' noncoding region. It does not contain a poly A termination or a canonical poly A initiation signal, which suggests that this 3' region is not complete (4). Clone 2-4 was sequenced in M-13 by the dideoxy method. Protein sequence, again, provided confirmation that the selected open reading frame was correct and did not divigate due to a skipped base. In addition, the protein sequence enabled us to establish that the clone likely encoded for the 11S species. We are sequencing the other clones based on initial findings, are optimistic that we have found a clone for another acetycholinesterase species. There is one caveat: The fact that all clones end in EcoR-1 sites suggests incomplete methylation in the library preparation. Clones 1-1, 1-9 and 1-10 are probably identical but reflect another gene of acetylcholinesterase. They are being sequenced. Clone 1-9, which is nearly 2.9 kb in length, is our candidate for obtaining complete 5' and 3' noncoding regions. Clone 2-9 is probably a shortened version of 1-9, terminating at the EcoR-1 site. In short, valuable information will continue to accrue as we compare c-DNA inferred sequences with actual protein sequences. Accordingly, the combination of molecular biology and protein chemistry should enable us to identify all of the structural polymorphisms in Torpedo acetylcholinesterase.

5. General aspects of acetylcholinesterase structure deduced from amino acid and nucleotide sequencing

All of the above data enable us to arrive at the following conclusions:

a. Acetylcholinesterase contains a hydrophobic leader sequence (residues -13-0) but contains no other hydrophobic domains which are candidates for membrane-spanning regions. This it is likely to be an exported protein and its membrane attachment site(s) arises as a consequence of posttranslational modifications.

b. The active center serine is at residue 200. The N-terminal location contracts with the serine proteases of similar size that function in the clotting cascade (i.e., factor IX and prothrombin).

c. No significant global or local homology is found with the acetylcholine receptor.

d. Although acetylcholinesterase is closely homologous to human butyrylcholinesterase, no significant global homology and very limited local homology are found with other serine proteases: the largest local homology is seen with liver aliesterase and the carboxylesterases.

e. Substantial homology is found between acetylcholinesterase and thyroglobulin in their C-terminal regions (acetylcholinesterase residues 1-575; thyroglobulin residues 2168-2750). Six of the eight cysteines are conserved, suggesting a similar folding pattern for the two macromolecules. The region between 160 and 190 shows greater than 60% identity.

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